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PRINCIPAL INVESTIGATOR: Yanis Boumber, MD, PhD

CONTRACTING ORGANIZATION: University of New Mexico
Albuquerque, NM 87131

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University of New Mexico Health
Sciences Center
MSC09 5220
1 University of New Mexico
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14. ABSTRACT `200 words-fit in the box`

Purpose, scope: The objective of this project is to expand our mechanistic data to characterize the functional roles of MSI2 in human NSCLC cells and test whether MSI2-overexpressing cells are more sensitive to γ-secretase and TGF-β receptor Type I kinase (TGF-βRI) inhibitors (Aim 1). I also aim to investigate if MSI2 expression is clinically predictive in tumor specimens from lung cancer patients (Aim 2). **Major findings and progress:** Depletion of MSI2 in multiple independent metastatic murine and human NSCLC cell lines reduced invasion and metastatic potential, independent proliferation effects. MSI2 depletion significantly induced expression of proteins associated with epithelial identity, including tight junction claudin proteins and down-regulated direct translational targets associated with EMT, and unexpectedly upregulated NOTCH pathways. Depletion of TGFβRI or SMAD3 resulted in reduced invasion, while overexpression of TGFβRI reversed the loss of invasion associated with MSI2 depletion. Interestingly, MSI2 depletion reduced E-cadherin expression, while increasing fibronectin (FN1), reflecting a mixed epithelial–mesenchymal phenotype. MSI2 provides essential support for TGFβR1/SMAD3 signaling, contributes to NSCLC progression and may be a predictive biomarker of NSCLC aggressiveness. TGF-βRI and γ-secretase drug studies in vitro and in vivo are ongoing, while immunohistochemistry studies are starting Fall 2016.

15. SUBJECT TERMS
Non-small cell lung cancer, invasion, metastasis, pro-invasive signaling, RNA binding proteins, Musashi, TGF-beta, epithelial mesenchymal transition (EMT), Notch, gamma-secretase, tissue microarrays (TMA).

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Table of Contents

Page

1. Introduction…….4

2. Keywords…….4

3. Accomplishments……...……...4

4. Impact……...……11

5. Changes/Problems...…..……11

6. Products……..……..…..…….11

7. Participants & Other Collaborating Organizations…….12

8. Special Reporting Requirements……14

9. References……14

10. Appendices……N/A

1. INTRODUCTION

Analyzing tumors from a mouse model of NSCLC, we identified upregulation of Msi2 as a previously unrecognized marker of invasion and metastasis in NSCLC. Msi2 knockdown in metastatic murine NSCLC cell lines decreased invasion and metastasis. Candidate pathway analysis and reverse-phase protein array screening identified EMT-associated proteins including the TGF-β receptor Type I (TGF-βRI), the Notch inhibitor Numb, and fibronectin, as strongly regulated by Msi2. An initial probe of 123 primary human NSCLC specimens, we have found that Msi2 is significantly elevated in tumors versus normal lung epithelium, suggesting relevance to physiological NSCLC in patients. The objective of this project is to expand our mechanistic data to characterize the functional roles of MSI2 in human NSCLC cells.

2. KEYWORDS

Non-small cell lung cancer, invasion, metastasis, pro-invasive signaling, RNA binding proteins, Musashi, TGF-beta, epithelial mesenchymal transition (EMT), Notch, gamma-secretase, tissue microarrays (TMA).

3. ACCOMPLISHMENTS

What were the major goals and objectives of the project?
Our preliminary data suggest the hypothesis that Msi2 is a master switch for invasion and metastasis that provides essential support for TGF-β and Notch-dependent oncogenic in a subset of metastatic NSCLC. The objective of this proposal is to expand our mechanistic data to characterize the functional roles of Musashi proteins in human NSCLC cells and to test whether Msi2 overexpressing cells are more sensitive to γ-secretase and TGF-β receptor Type I kinase (TGF-βRI) inhibitors (Aim 1): as a developing physician-scientist, my bigger goal would then be to develop a Phase I/II trial focused on evaluation of these inhibitors using Msi2 as a biomarker for response. I also aim to investigate if the expression of Msi2 is clinically predictive in tumor specimens from lung cancer patients (Aim 2). A functional role for Msi proteins has never been identified or studied in NSCLC; of the two Msi proteins, has almost all work has focused on Msi1. I hope these studies of Msi2 will improve understanding of what drives NSCLC, and suggest improved treatment strategies.

Aim 1: Evaluate the functional role of Msi2 in human lung cancer cell lines. Human and murine cell lines with depleted or overexpressed Msi2 will be used for functional assays in vitro and orthotopic xenograft studies in mice. The functional role of Msi2 in regulating Numb/Notch, TGF-β and markers of epithelial-mesenchymal transition (EMT) in NSCLC pathogenesis will be characterized. Msi2 manipulated lung cancer cells will be treated with γ-secretase and TGF-β receptor Type I kinase (TGF-βRI) inhibitors, to determine if those are differentially sensitive to these drugs, and that treatment with these agents will reduce tumor invasion and metastasis.
Overall, 65% completed.

Subtask 1: Confirmation of Msi2 function in human NSCLC models. Cell lines: H358, A549; H441, H322, 293T (MD Anderson / ATCC). *SA1:* Cell proliferation assays: second method of measuring proliferation, performing automated counting DAPI-stained nuclei using high throughput equipment in the institutional translational science facility. 1-6 months. 100% completed.
Subtask 2: Validation of functional significance of Msi2-dependent signaling effectors. Cell lines used: 344SQ, A549, H358, H441, H322, 293T (MD Anderson / ATCC). 1-18 months. 100% completed.
Subtask 3: Determination of the role of Msi2 in NSCLC response to Notch and TGF-β targeting drugs (in SCID mice). Cell lines used: H358, A549 (MD Anderson / ATCC). 12-24 months. 40% completed.

Aim 2: Establish how Msi2 expression predicts tumor phenotypes, patient outcomes, and invasion-related signaling in primary human NSCLC tumors. We will analyze lung cancer tissue microarrays for Msi2 expression

relative to normal lung tissues, and Msi2 correlation with stage, grade, and survival in lung cancer patients. We will determine whether Msi2 expression correlates with expression of Numb, Notch pathway activity, TGF-β R1 and SMAD3 expression, and therapeutic response.
Overall, 5% completed.
Subtask 1: Obtain UNM SRC and FCCC Committee Approvals for the construction and use of TMAs. 1-3 months. 100% completed.
Subtask 2: Analyze expression of Numb, activated Notch, and HES1, TGF-βRI, phosphorylated SMAD3 and correlate it with Msi2 expression and patient and tumor data in lung cancer tissue microarrays (UNMHSC), using Aperio Scan Scope CS. 12-22 months. 0% completed.
SA1: Msi2 Specificity control experiments on the TMAs using MSI2-knockdown vs control cell lines that will be paraffin embedded and stained in parallel manner to TMA. In addition, we will use 2d alternative Msi2 antibody to validate our data if needed (Abcam #ab50829, or ab156770).12-22 months. 0% completed.

Generally, the goals will not change from one reporting period to the next and are unlikely to change during the final reporting period. However, if the awarding agency approved changes to the goals during the reporting period, list the revised goals and objectives. Also explain any significant changes in approach or methods from the agency approved application or plan.

What was accomplished under these goals?
Major activities for this project as per major goals outlined above included an extensive series of experiments as outlined in (3) below and required applications of various molecular, cancer biology and cell biology experimental procedures, and included primarily in vitro and cell culture approaches, with currently ongoing animal experiments which have not yet resulted, being performed in mice.

Specific objectives, once again, included expanding our mechanistic data to characterize the functional roles of Musashi proteins in human NSCLC cells and to test whether Msi2 overexpressing cells are more sensitive to γ-secretase and TGF-β receptor Type I kinase (TGF-βRI) inhibitors (Aim 1): as a developing physician-scientist, my bigger goal would then be to develop a Phase I/II trial focused on evaluation of these inhibitors using Msi2 as a biomarker for response. I also aim to investigate if the expression of Msi2 is clinically predictive in tumor specimens from lung cancer patients (Aim 2). A functional role for Msi proteins has never been identified or studied in NSCLC; of the two Msi proteins, has almost all work has focused on Msi1.

Significant results and major findings. The narrative and 8 corresponding figures below generally match Aim 1 of the grant, which has been 65% completed. Sub-Aim 3 experiments (“mouse trials”) are nearly half done, and

remaining experiments are currently ongoing.***MSI2 depletion in metastatic NSCLC cells inhibits invasion in vitro and has minimal effect on proliferation in NSCLC.*** Based on expression profiles of NSCLC cell lines available through the Cancer Cell Line Encyclopedia[1], we identified the human NSCLC cell lines A549 ($KRAS^{mut}$) and H358 ($KRAS^{mut}$;$TP53^{-/-}$) as metastasis-competent adenocarcinoma cell lines with high expression of MSI2. We previously used shRNA depletion of these and two metastatic murine NSCLC cell lines (344SQ and 531LN2) to further study the role of MSI2 in metastasis. Similar results were obtained using transient siRNA transfections to deplete MSI2 as well, which we recently published (Kudinov et al[2]). To complement these studies, we now generated two additional MSI2-depleted and control human cell lines, A549 and H358 (Figure 1A). MSI2 depletion consistently and significantly reduced invasion through Matrigel for all lines, including A549 and H358 cell line models (Figure 1 B, C). As indicated in the CDA, we also analyzed cell proliferation upon MSI2 depletion. For three of the four cell lines (344SQ, A549, and H358), depletion of MSI2 had no effect on cell proliferation in vitro (Fig 1 L, M), using both CellTiterBlue (CBT) and complementary DAPI cell counting as was requested by the DOD CDA grant reviewers per SA1 goals.

In a reciprocal approach, as outlined in Aim1 of the proposal, we recently overexpressed MSI2 in cell lines with low MSI2 levels. We initially planned to overexpress MSI2 in H441 and H322 cell lines. However, we were unable to reliably overexpress MSI2 in H441 cell line, and therefore we used an alternative approach by overexpressing MSI2 in 393P murine cell line. Later, we were able to successfully overexpress MSI2 in H322 cell line as well. In view of these slight, likely technical, changes, we first generated functional data for the 393P cells, and we have recently generated the H322 cell lines, control and MSI2-overexpressing lines (Fig. 5 H; functional

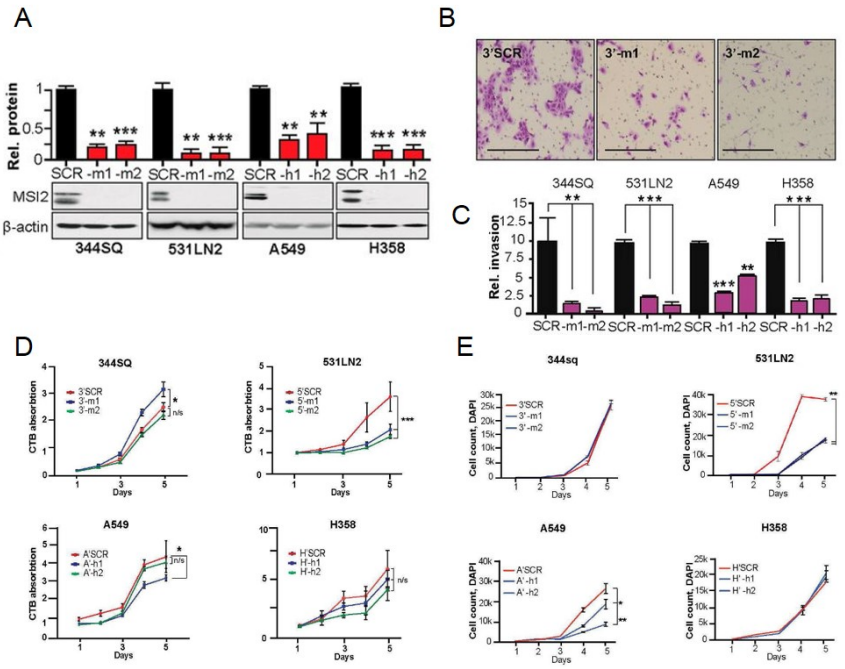

Fig. 1. Elevated MSI2 expression supports NSCLC invasion with minimal effects on proliferation. (A) Western validation of MSI2 depletion with two independent shRNAs (–m1, –m2, –h1, and –h2) in indicated cell lines, relative to control shRNA (SCR)-depleted cells. (B, C) Quantified (H) and representative (I; for 344SQ cells) data for invasion for models shown (D, E) proliferation results for indicated cell lines using CBT (D) and DAPI (E).

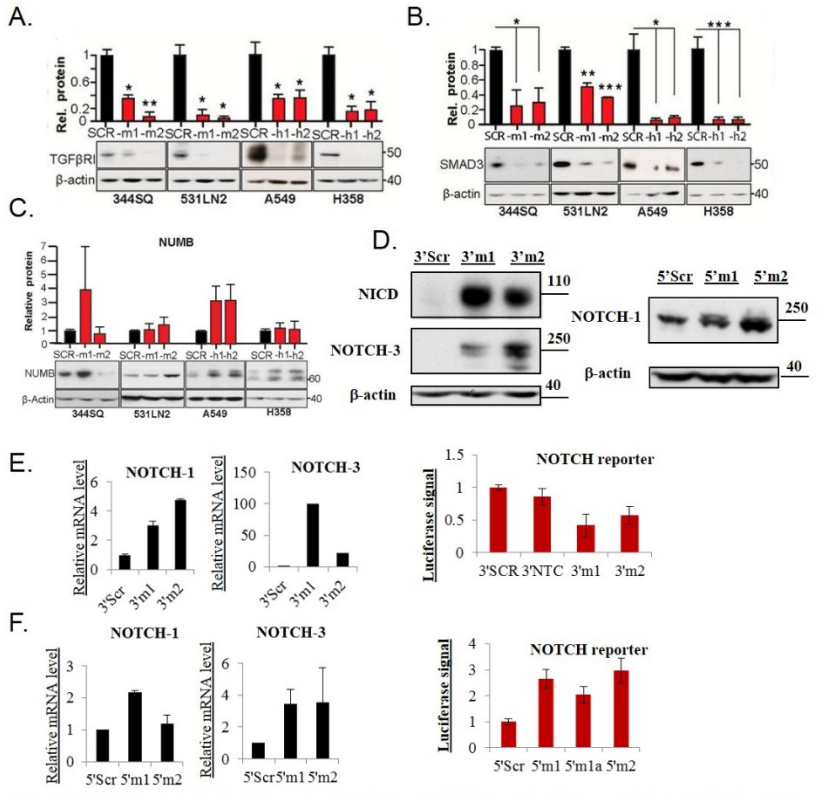

Fig. 2. MSI2 depletion controls the expression of TGFbR1, SMAD3 and NOTCH pathway proteins. (A, B and C) Western analysis of expression of TGFβR1 (A) and SMAD3 (B) and NUMB (C) in murine and human NSCLC cell lines with stably depleted MSI2. Graphs represent data from 3 independent runs. For all graphs,*P < 0.05, **P < 0.01, and ***P < 0.001 relative to SCR (scrambled shRNA) controls. (D) Western analysis of expression of Notch pathway –related proteins in 344SQ and A549 MSI2-expressing (3'SCR, 5'SCR) and MSI2-depleted (3' and 5' m1, m2) cells (E, F) RT-PCR expression of NOTCH pathway related genes in 344SQ (E) and A549 (F) MSI2-expressing (3'SCR, 5'SCR) and MSI2-depleted (3' and 5' m1, m2) cells.

proliferation and invasion data for H322 cell line are currently pending/ ongoing). In 393P non-metastatic cells, which have low endogenous levels of Msi2. MSI2 overexpression did not affect 393P proliferation (Fig 4A), but greatly increased invasion through Matrigel (Fig 4B, C). Finally, analysis of migration independent of invasion (Kudinov et al[2]) showed limited effects of MSI2 depletion. We therefore focused subsequent analysis on mechanistic analysis of invasion-related signaling.

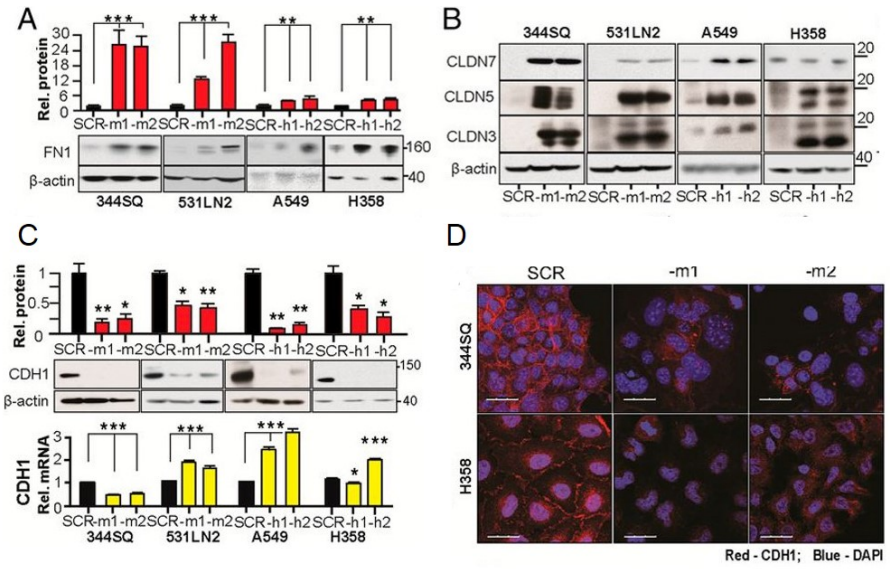

Fig. 3. MSI2 depletion controls the expression of indirect and direct targets relevant to invasion and EMT. (A and B) Western analysis of FN1 (A) and CLDN7, CLDN5, and CLDN3. (B) In murine and human NSCLC cell lines in the context of MSI2 depletion with independent targeting shRNAs (–m1/–m2, –h1/–h2). SCR, control scrambled shRNA. Graphs represent data from four independent runs .) (C) Western (Top) and qRT-PCR (Bottom) analysis of E-Cadherin expression in 4 NSCLC cell line models with / without depleted MSI2. Graphs represent data from 3 independent runs. For all graphs, *P < 0.05, **P < 0.01, and ***P < 0.001 relative to SCR (scrambled shRNA) controls. (D) Immunofluorescence analysis of E-Cadherin staining in 344SQ murine (Top) and H358 human (Bottom) NSCLC cell lines, with or without depleted MSI2. Blue, DAPI; red, E-Cadherin. (Scale bars, 30 μm).

Candidate-based and unbiased investigation of MSI2-regulated signaling.

Direct MSI2 translational targets defined in other cell types that might be relevant to the invasiveness of NSCLC cells and tumors include the TGF-β receptor (TGFβR1) and its effector SMAD3[3], which promote epithelial-mesenchymal transition (EMT) by downregulating E-cadherin (CDH1) and inducing other transcriptional changes[4]. We found that stable or transient MSI2 knockdown caused strong downregulation of TGFβR1 and SMAD3, predominantly at the protein level, in all 4 models (Fig 2A, B). Reciprocally, exogenous overexpression of MSI2 induced TGFβR1 and SMAD3 expression in 393P, H322 cells and caused loss of CLDN3, CLDN5 and CLDN7 expression in the 393P cell line (Fig 5 D, E, H).

While some studies suggested expression of the NOTCH regulator NUMB, is influenced by MSI1/2[5], we found no consistent and significant differences in NUMB in MSI2-depleted cells (Fig S6D), supporting the idea that regulation of NUMB by MSI1/2 may depend on cellular context[3, 6]. To investigate NOTCH pathways signaling in more depth, we tested for NOTCH-3 / NOTCH-1 RNA and protein expression in 344SQ and 531LN2 cells (NICD protein level was tested in 344SQ cells as well), Fig. 2 D-F. Surprisingly, this data demonstrated an upregulation of NOTCH pathway upon MSI2 depletion. Luciferase reporter data using RBP-Jk reporter (Qiagen) in 344SQ and 531LN2 cells has shown

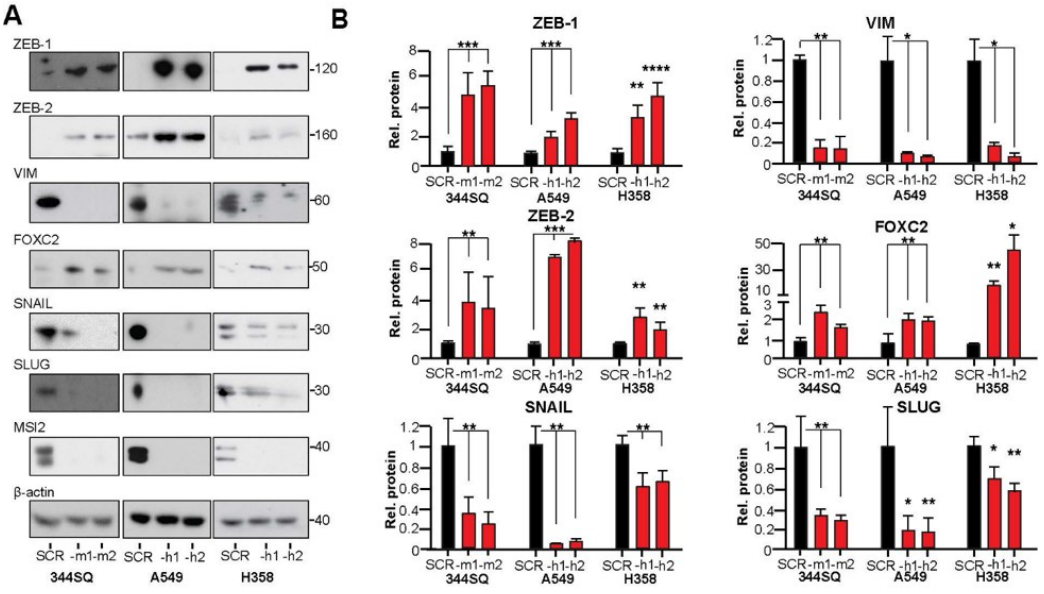

Fig. 4. MSI2 depletion: discovery of additional relevant MSI2-regulated EMT proteins. Western blot analysis (**A**) and quantification (**B**) of MSI2, ZEB-1, ZEB-2, FOXC2, SNAIL, SLUG, VMN (vimentin) versus beta-actin loading control in 344SQ, A549 and H358 cell lines expressing MSI2 (SCR) or depleted of MSI2 (-m1, -m2, -h1, -h2).

inconsistent results which varied depending on cell line (Fig. 2 E-F).

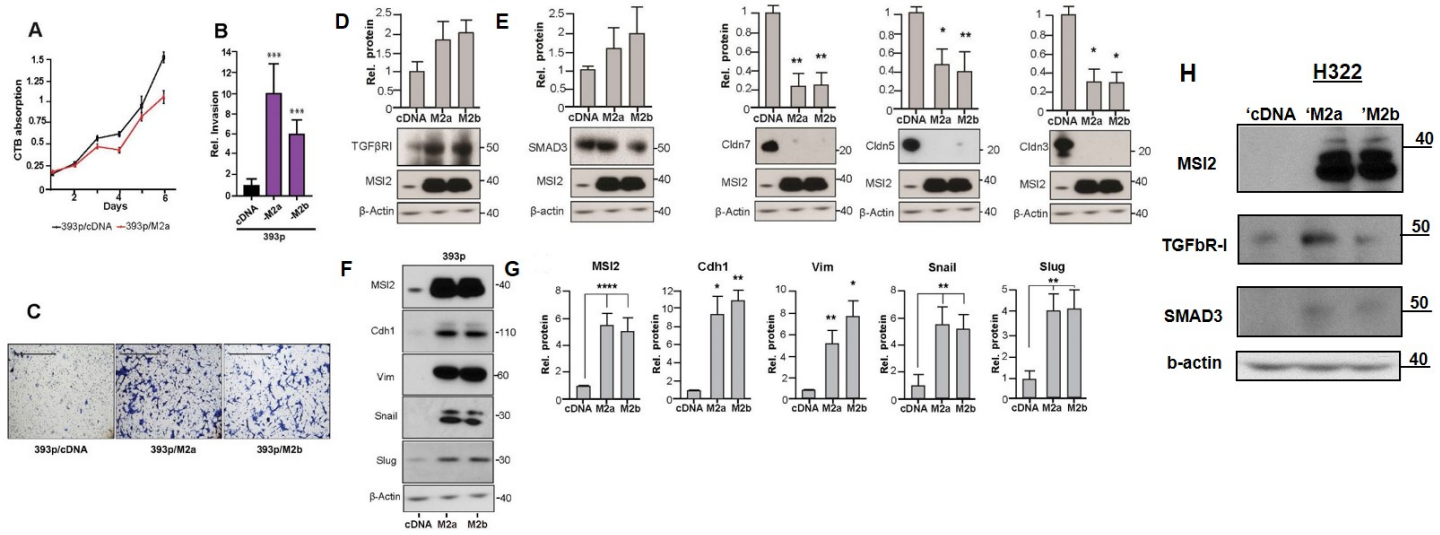

Fig. 5. MSI2 overexpression: functional and signaling effects validation of MSI2 effects in 393P cell line. A. Quantification of CellTiterBlue (CTB) proliferation assays of 393p/M2a and 393p/M2b MSI2-overexpressing clones vs 393p/cDNA control cell lines. **B.**, **C**. Quantification (**B**) and representative image (**C**) of Matrigel invasion analysis of 393p/cDNA control and 393p/M2a and 393p/M2b MSI2 overexpressing cell lines. **D, E.** Western blot analysis of indicated proteins in the 393P cell line, overexpressing MSI2 (393p/M2a and 393p/M2b versus the 393p/cDNA control cell line). All graphs: *, p≤0.05; *, p≤0.01; ***, p≤0.001 relative to controls. **F., G.** Western blot analysis (C) and quantification (D) of MSI2, E- Cadherin, vimentin, SLUG and SNAIL protein expression 393p/cDNA control and 393p/M2a and 393p/M2b MSI2 overexpressing cell lines. All graphs: *, p≤0.05; *, p≤0.01; ***, p≤0.001 relative to controls. **H.** Western blot analysis of indicated proteins in the H322 cell line, overexpressing MSI2 (H322/M2a and H322/M2b versus the H322/cDNA control cell line).

We previously used Reverse Protein Phase Array (RPPA) to query 171 total and phospho-proteins for expression changes associated with Msi2 knockdown using control shRNA and Msi2-targeted shRNA derivatives of 344SQ cells[2]. This work suggested a number of novel candidates associated with Msi2 expression and relevant to control of EMT and invasion. Proteins with the greatest magnitude of response to Msi2 depletion that were subsequently validated by low throughput Western analysis included the tight junction (TJ)-associated protein claudin 7 (CLDN7)[7-9], elevated 19.4-fold, and the ECM protein fibronectin (FN1)[10-13], elevated 2.5-fold[2]. Subsequent independent evaluation confirmed these RPPA results, as MSI2 depletion significantly elevated FN1 mRNA (4.0-9.4-fold) and protein (2.4-23 fold) in all 4 cell lines. While initially not planned, we expanded investigations of the EMT-related claudin proteins, and we indeed found elevated CLDN7 protein (2.5-28 -fold) in 3 of the 4 cell lines (Fig 3B). Results were independently confirmed using transient siRNAs to deplete MSI2 (Kudinov et al[2]). NSCLC cells have been shown to express multiple claudins with partially redundant function[14], most not represented in the RPPA panel. In direct testing, we found MSI2 depletion also induced CLDN3 and CLDN5 in all 4 cell lines at the protein level (3.8-22 fold for CLDN3 and 3.4-41 fold for CLDN5) (Fig 3B), making restraint of claudin expression a consistent feature of MSI2 function. Studies of the MSI proteins (predominantly focused on MSI1) have defined these proteins as RNA-binding proteins that regulate mRNA translation[3, 15, 16]. The induction of claudins may reflect a combination of transcriptional and post-transcriptional consequences of MSI2 depletion, as the mRNA level shows induction less marked than at the protein level (Kudinov et al[2]). However, the claudin mRNAs lack [(G/A)U(n)AGU (n = 1–3)] consensus motifs for MSI2 binding described in Wang et al[17], suggesting direct regulation of translation is not involved.

Depletion of MSI2 affects the composition of cell-cell junctions and causes partial EMT. Based on the action of MSI2 in supporting the expression of TGFβR1 and SMAD3, while repressing CLDN3, CLDN5, CLDN7, and FN1, we hypothesized that the reduced invasiveness of MSI2-depleted cells might reflect changes involving TJs and reduced EMT, associated with elevated E-cadherin (CDH1). Unexpectedly, total epithelial protein E-cadherin protein expression was decreased by MSI2 depletion, while mRNA levels were not consistently affected (Fig. 3C); immunofluorescence analysis confirmed that expression of E-cadherin at cell-cell junctions was much reduced by MSI2 depletion (Fig 3D). In contrast, there was a significant increase in CLDN3 and

CLDN7 staining at cell-cell contact points, while TJP1 (ZO-1), which localizes to the cytoplasmic surface of TJs, was unaffected (Kudinov et al[2]).

Based on the effects of MSI2 in regulating EMT-related signaling, we examined expression of additional proteins associated with mesenchymal identity (Fig 4A, 4B). MSI2 depletion upregulated the pro-EMT factors ZEB1, ZEB2 and FOXC2 but downregulated VIM (vimentin), SLUG and SNAIL. Conversely, MSI2 overexpression in 393P cells induced CDH1, VIM, SNAIL and SLUG (Fig 5F, G). Collectively, these data indicated a mixed effect of MSI2 depletion on EMT.

MSI2 regulation of invasion via TGFβR1, SMAD3 and CLDN7. To assess the functional interaction between MSI2, its direct targets TGFβR1 and SMAD3, and claudins, we depleted SMAD3 or TGFβR1 in MSI2-depleted versus control cell lines. SMAD3 knockdown reduced CDH1 expression levels in both parental and MSI2-depleted lines (Fig 6A, B). By contrast, the relationship between TGFβR1 and CDH1 expression was modulated by MSI2 status, with the TGFβR1 knockdown elevating CDH1 expression in the parental cell lines, but reducing it in MSI2-depleted cell lines (Fig 6A, B). Importantly, depletion of TGFβR1 or SMAD3 caused a statistically significant decrease in invasion in SCR-depleted NSCLC cell lines, but not in those with depleted MSI2 (Fig 5C). Conversely, overexpression of TGFβR1 partially but incompletely rescued the decrease in invasion seen in MSI2-depleted cells (Kudinov et al[2]), suggesting other contributing factors.

The profile of mixed pro- and anti-EMT changes, and incomplete rescue by TGFβR1 overexpression, suggested a possible important role for claudin-associated TJs in limiting NSCLC invasion induced by MSI2-dependent TGFβR1/SMAD3 signaling. Exploring the relationship between these proteins, we found that siRNA depletion of TGFβR1 or SMAD3 did not significantly affect the expression of CLDN3, CLDN7, or MSI2. This indicated that MSI2 regulates CLDN3/CLDN7 expression independently of TGFβR1 and SMAD3 (Fig 6A, B). Importantly, depletion of TGFβR1 or SMAD3 caused a statistically significant decrease in invasion in SCR-depleted NSCLC cell lines, but not in those with depleted MSI2 (Fig 6 C).

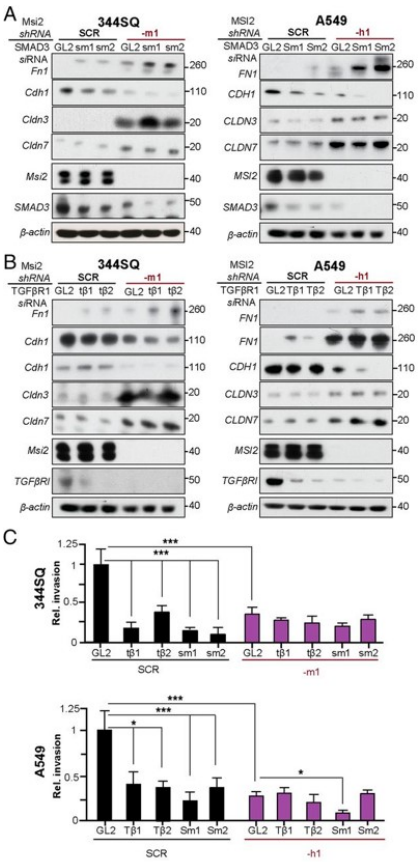

Fig. 6. Functional interaction of MSI2 with TGFβR1, SMAD3, FN1, E-Cadherin, and CLDN3 and CLDN7. (A) Western for expression of indicated proteins in the 344SQ and A549 cell lines with (–m1 and –h1) or without (SCR) shRNA depletion ofMSI2, and with (–sm1, –sm2, and –Sm1, –Sm2) or without (GL2) siRNA depletion of SMAD3. (B) Western for expression of indicated proteins in the 344SQ, A549 cell lines with (m1 and h1) or without (SCR) shRNA depletion of MSI2, and with TGFβR1 (–tβ1, –tβ2 and –Tβ1, –Tβ2) or without (GL2) siRNA depletion of TGFβR1. (C) Quantification of results from 3 independent Matrigel invasion assays for 344SQ and A549 with (–m1 and –h1) or without (SCR) shRNA depletion of MSI2, in the context of additional siRNA depletion of TGFβR1 (–tβ1, –tβ2 and –Tβ1, –Tβ2) or SMAD3 (–sm1, –sm2 and –Sm1, –Sm2) vs siRNA negative control (GL2). *P < 0.05 and ***P < 0.001 relative to controls.

Determination of the role of Msi2 in NSCLC response to Notch and TGF-β targeting drugs.

We hypothesized that MSI2 expression can affect drug responses.

Here, we also initiated experiments to determine whether Msi2 expression conditions response to γ-secretase and TGF-β receptor Type I kinase (TGF-βRI) inhibitors, as predicted by its regulation of NUMB/Notch and TGFβ/SMAD2/3 pathways. We performed IC50 determinations, as well as invasion assays as described in preliminary data, for drugs used in clinical trials against solid tumors. We will assess RO-4929097, a small molecule γ-secretase inhibitor which blocks Notch signaling[48], and LY2157299[17, 18], a small molecule inhibitor of the TGF-βRI kinase activity, in Msi2-manipulated versus control cells. We used A549 and H358 human NSCLC cell lines with endogenous or transiently / stably depleted Msi2, or overexpressed Msi2. Briefly, these cell lines will be plated in 96 well plates; 24 hours after plating, drugs or vehicle will be added, and 72 hours later, cells will be analyzed by CellTiterBlue for reduction in proliferation. As described in Figure 7 (A, B), preliminary in vitro results in 344SQ and A549 cell lines demonstrated increased sensitivity of MSI2-depleted cells (3'74, 3'75 and A'9, A'11) to RO-4929097 gamma-secretase compound. In contrast, we did

not observed significant difference in IC50 curves between MSI2-expressing or MSI2-depleted 344SQ cells treated with LY2157299 TGFbR1 inhibitor. In vivo mouse experiments are currently pending. LY2157299 mouse trials using A549, H358 MSI2-manipulated cell line xenografts are about 70% completed, while RO-4929097 mouse experiments are planned for the Fall of 2016.

Taken together, we conclude that MSI2 stimulates invasion in lung cancer in part by sustaining TGFβR1 signaling and suppressing the expression of CLDN7 and potentially other claudins. Our study for the first time shows that elevation of MSI2 expression progressing NSCLC supports tumor cell invasion and metastasis by modulating TGFβ- dependent EMT, and repressing claudin expression (Fig. 8).

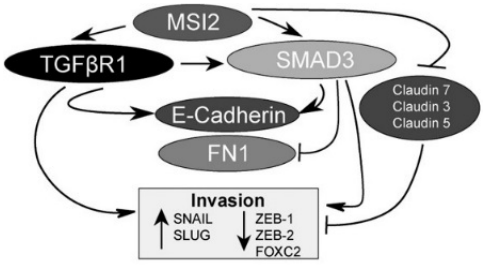

Fig. 8. Model for MSI2 action in coordinating EMT and invasion potential.

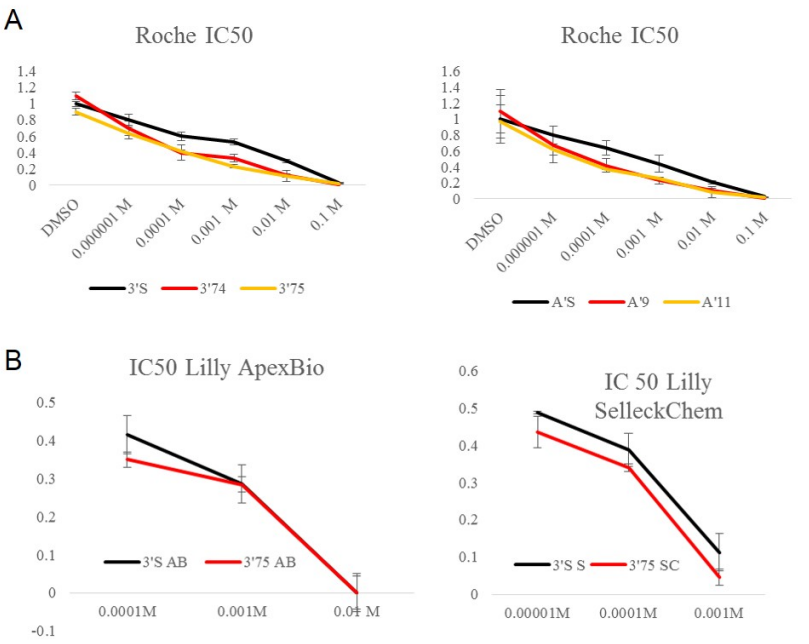

Fig. 7. IC50 curves for gamma-secretase and TGFbR1 inhibitors in NSCLC cell lines. (A) Roche gamma-secretase inhibitor IC50 curve shows increased sensitivity of MSI2-depleted cells to the compound relative to control (3'S) in 344SQ and A' S in A549 (B) Lilly TGFbR1 inhibitor shows similar activity against MSI2-expressing (S) and MSI2-depled (75) cells in 344SQ cell line.

What opportunities for training and professional development has the project provided?

I have attended 2016 AACR where MSI2 data poster was successfully presented. I have interacted with several scientists working on RNA-binding proteins in the USA and Asia.

How were the results disseminated to communities of interest?

Our MSI2 PNAS publication sparked significant interest in scientific community. This was illustrated by a feature report in a weekly publication, June 23, 2016 BioCentury Innovations. BioCentury Inc. was founded in 1992 by David Flores, President and CEO, and Karen Bernstein, Ph.D., Chairman. This is a "first-in-class" biotech-focused business journal that provides independent, authoritative intelligence built on in-depth and accurate reporting. Their mission is to identify and communicate the essential scientific, business, financial and public policy actions required to successfully bring progressive medical solutions to patients. *BioCentury Innovations* (formerly SciBX) specifically identifies commercially promising translational science and assesses the next steps required to develop the technology.

In addition, press release is planned for October 2016 at Fox Chase Cancer Center web-site, to feature MSI2 NSCLC story as one of the innovative scientific breakthroughs at the Center.

What do you plan to do during the next reporting period to accomplish the goals?

We plan to complete the remaining experiments for the Aim 1 (mouse trials using RO-4929097 and LY2157299 compounds, including HE staining of mouse xenograft A549, H358 tumors, and data analysis). We also plan to complete the Aim 2 entirely. Completion of Aim 2 will involve analysis of the human tissue microarrays for signaling proteins supported by MSI2. These specimens will be stained and analyzed for the expression of Ki-67, TGF-βRI, phosphorylated SMAD3 and Numb. Expression of activated, cleaved Notch will also be measured, and

Hes1 expression will be used as an endpoint indicator of Notch pathway activity. TMAs will be analyzed for correlation of Msi2 expression with pathologic stage, lymph node status, presence of metastasis, grade. We will determine whether Msi2 expression correlates with expression of Numb, activated Notch, and HES1, TGF-βRI, phosphorylated SMAD3, as predicted by in vitro studies.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
MSI2 work we published has advanced the field of RNA-binding proteins biology. In particular, it is the first work to describe MSI2 role in driving NSCLC progression. It was also the first report to describe regulation of claudins by MSI2, which may have important implications of understanding how NSCLC develops.

What was the impact on other disciplines?
Our findings may indirectly affect pharmacology and drug development fields. Since NSCLC is being driven by MSI2, our publication may spark an increase interest in developing MSI2 inhibitors for cancer therapy.

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?
I believe that our project that focuses on lung cancer may draw additional interest from general public in understanding and awareness of lung cancer burden and impact on patients around the country.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change
No changes anticipated, nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
No delays anticipated, nothing to report

Changes that had a significant impact on expenditures
Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

6. PRODUCTS

Publications, conference papers, and presentations:
Journal publications
Kudinov AE, Deneka A, Nikonova AS, Beck TN, Ahn YH, Liu X, Martinez CF, Schultz FA, Reynolds S, Yang DH, Cai KQ, Yaghmour KM, Baker KA, Egleston BL, Nicolas E, Chikwem A, Andrianov G, Singh S, Borghaei H, Serebriiskii IG, Gibbons DL, Kurie JM, Golemis EA, Boumber Y. Musashi-2 (MSI2) supports TGF-β signaling and inhibits claudins to promote non-small cell lung cancer (NSCLC) metastasis. **Proc Natl Academy Science** U S A. 2016 Jun 6; PubMed PMID: 27274057.
Acknowledgement of federal support (yes)

Alexander Kudinov, Alexander Deneka, Anna Nikonova, Ilya Serebriiskii, Tim N. Beck, Qi Cai, Brian L. Egleston, Emmanuelle Nicolas, Hossein Borghaei, Don Gibbons, Jonathan Kurie, Erica A. Golemis and Yanis

Boumber. "*Musashi-2 (MSI2) drives TGFBR1/SMAD3 dependent partial EMT and supports VEGFR2 expression and metastasis of human and mouse NSCLC cells.*" AACR 2016 abstract # 1584. Proceedings: AACR 105th Annual Meeting; Apr 12-22, 2015; New Orleans, LA. Published.
Acknowledgement of federal support (yes)

Books or other non-periodical, one-time publications
Nothing to report

Other publications, conference papers, and presentations
Alexander Kudinov, Alexander Deneka, Anna Nikonova, Young-Ho Ahn, Xin Liu Liu, Ilya Serebriiskii, Andrey Efimov, Dong-Hua Yang, Mark Andrake, Emmanuelle Nicolas, Brian Egleston, Hossein Borghaei, Don Gibbons, Jonathan Kurie, Erica Golemis and Yanis Boumber. *"Musashi-2 (MSI2) activates TGF-β signaling and inhibits CLDN7 to promote non-small cell lung cancer (NSCLC) metastasis."* Presented at AACR 104th Annual Meeting; Apr 12-22, 2015; Philadelphia, PA.
Acknowledgement of federal support (yes)

Website(s) or other Internet site(s)
Nothing to report

Technologies or techniques
Nothing to report

Inventions, patent applications, and/or licenses
Nothing to report

Other Products
We have generated additional MSI2-depleted (A549, H358) or overexpressing (H322, 393P) cell lines, which are useful research tools to study MSI2. While those are currently only used in our laboratory, we are open to sharing it with scientific community (independent labs or investigators, Addgene, other sources).

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Name: **Alexander Kudinov, MD**
Project Role: Postdoctoral fellow
Researcher Identifier (e.g., ORCID ID): N/A
Nearest calendar month worked: 6
Contribution to Project: Dr. Kudinov performed the majority (~80%) of experiments for this project and performed at least half data analysis (see significant results and major findings and PNAS paper).
Funding Support: UNM Cancer Center Support Grant (NIH, P30 CA118100) developmental funds.

Name: **Yanis Boumber, MD, PhD**
Project Role: PI
Nearest calendar month worked: 5
Researcher Identifier (e.g., ORCID ID): N/A
Contribution to Project: Dr. Boumber performed some (~5-10%) of the preliminary experiments for this project (see Significant results and major findings and PNAS paper) and supervised the project, key experiments, and wrote PNAS paper
Funding Support: Institutional Funds

Name: **Erica Golemis, PhD**
Project Role: PI
Nearest calendar month worked: 1
Researcher Identifier (e.g., ORCID ID): N/A
Contribution to Project: Dr. Golemis supervised the project, key experiments, and wrote PNAS paper
Funding Support: R21 CA181287 and R01 CA063366 (to EAG); NIH Core Grant CA006927 (to Fox Chase Cancer Center).

Name: **Alexander Deneka, MD**
Project Role: Graduate student
Researcher Identifier (e.g., ORCID ID): N/A
Nearest calendar month worked: 1
Contribution to Project: Dr. Deneka performed the several key (~15%) experiments for this project, including the majority of animal studies which have been completed, and also assisted with data analysis (see significant results and major findings of this report, and PNAS paper).
Funding Support: R21 CA181287 and R01 CA063366 (under EAG); and by the NIH Core Grant CA006927 (to Fox Chase Cancer Center). Russian Science Foundation project 15-15-20032 (to AD).

Name: **Anna Nikonova, PhD**
Project Role: Postdoctoral fellow
Researcher Identifier (e.g., ORCID ID): N/A
Nearest calendar month worked: 1
Contribution to Project: Dr. Nikonova some of the experiments (5%) and some data analysis for this project and also supervised Dr. Kudinov and Dr. Deneka in some key experiments, including animal experiments and Westerns
Funding Support: R21 CA181287 and R01 CA063366 (under EAG); and by the NIH Core Grant CA006927 (to Fox Chase Cancer Center).

Name: **Brian Egleston, PhD**
Project Role: Statistician
Researcher Identifier (e.g., ORCID ID): N/A
Nearest calendar month worked: 1
Contribution to Project: Dr. Egleston performed some key statistical analysis for this project, and supervised Dr. Kudinov and Dr. Deneka in data analysis (see Significant results section of this report and PNAS paper).
Funding Support: by the DOD CDA (current award), P30 CA006927 grant to Fox Chase Cancer Center biostatistics department, and by the NIH Core Grant CA006927 (to Fox Chase Cancer Center).

Name: **Helen Hathaway, PhD**
Project Role: Collaborator
Researcher Identifier (e.g., ORCID ID): N/A
Nearest calendar month worked: 1
Contribution to Project: Dr. Hathaway is a Director of Animal facility at UNMCC. She helped design and implement animal protocols and mice experiments (intra-thoracic injections, gavages) and supervised Laura Laidler, Alexander Kudinov and Helen Nordquist in ongoing animal experiments (some of these data are being analyzed).
Funding Support: NIH, P30 CA118100 UNM Cancer Center Core Grant – Animal Modeling Shared Resource

Name: **Laura Laidler, MS**
Project Role: technician, animal experiments
Researcher Identifier (e.g., ORCID ID): N/A

Nearest calendar month worked: 1
Contribution to Project: Dr. Laidler performed some key animal experiment for this project, including ongoing intra-thoracic injections, gavages, drug studies in mice, and supervised Dr. Kudinov and Ms. Nordquist animal work (some of these data are being analyzed).
Funding Support: NIH, P30 CA118100 UNM Cancer Center Core Grant – Animal Modeling Shared Resource

Name: **Helen Norquist, BS**
Project Role: undergraduate student, animal experiments assistant
Researcher Identifier (e.g., ORCID ID): N/A
Nearest calendar month worked: 4
Contribution to Project: Ms. Nordquist performed some animal experiment for this project, including assistance with intra-thoracic injections, gavages, drug studies in mice (some of these data are being analyzed).
Funding Support: Dr. Boumber's UNM Start-up funds.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to report

What other organizations were involved as partners?
Fox Chase Cancer Center – academic institution and a major cancer center
-provided statistical support, and overall guidance, collaboration and supervision for the project, computer and lab equipment

UNM Comprehensive Cancer Center – academic institution
-provided financial support and collaboration, computer and lab equipment

8. SPECIAL REPORTING REQUIREMENTS

None

9. REFERENCES

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10. APPENDICES

N/A.